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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

INVENTOR(S)

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☐ Additional inventors are being named on the ____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

METHOD FOR PREPARING TISSUE CONSTRUCTS

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ENCLOSED APPLICATION PARTS (check all that apply)

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☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27

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Respectfully submitted,

SIGNATURE

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14.

METHOD FOR PREPARING TISSUE CONSTRUCTS

TECHNICAL FIELD

[0001] The invention relates to tissue engineering. It also relates to *in vitro* production of bioengineered tissue constructs containing spatially separated populations of living cells with extracellular matrix, and method of preparation thereof. This method can be applied to the construction of a wide range of tissue constructs requiring spatially distinct domains with specific cell populations, extracellular matrix and mechanical properties.

BACKGROUND OF THE INVENTION

[0002] Tissue engineering is the art of reconstructing tissue from isolated cells and extracellular matrix components. One efficient way of reconstructing tissues and organs is to first produce living tissue sheets. These sheets, composed of living cells and extracellular matrix, can be used as a starting material for the crafting of tissue-engineered constructs (4). When mechanically assembled with the appropriate tensile strength and the desired shape, the cells will continue to secrete matrix proteins and adjacent sheets will fuse together to form a solid structure (5,6,7). Moreover, these constructs can be made from autologous cells, thus avoiding the immune rejection of the graft.

[0003] Living tissue sheets sufficiently strong to be manipulated can be obtained by several methods. The extracellular matrix in the tissue sheet can be either exogenously added or self-produced by the cells. Cells can be seeded along with exogenous extracellular matrix components to form a tissue sheet (ref Bell). Also, cultured cells can synthesize their own extracellular matrix when incubated in the presence of the appropriate reagents (1). It has long been known that ascorbate stimulates collagen production in cultured cells of mesenchymal origin (1-3). When cultured on a plastic surface in the presence of ascorbic acid, fibroblasts and smooth muscle cells will form sheets that detach from the culturing substratum. This auto-assembly method yields tissue with organ-like mechanical properties and devoid of any synthetic material, making it perfectly compatible with the living organism in which it is to be implanted.

[0004] When a tissue reconstructed with layers of different cell types has to be produced, the successive assembly of the different separated sheets increase the production time of the final product. Furthermore, tissue artifacts may appear at the point of fusion between the sheets as prepared in the art.

SUMMARY OF THE INVENTION

[0005] One object of the present invention is to provide a method for assembling living tissue sheets for forming a continuous tissue construct comprising the step of:

a) providing at least two cell populations capable of forming at least two separated living tissue sheets, the cell populations being partially or totally confluent; and

b) causing edge contact between the cell populations of step a) for a period of time sufficient for assembling the living tissue sheets into a single continuous tissue construct.

[0006] The cell populations of step a) may be composed of homologous or heterologous types of cells and are preferably mammalian cells selected from the group consisting of mesenchymal cells, muscle cells, smooth muscle cells or fibroblasts.

[0007] Another object of the present invention is to provide single continuous tissue construct composed of at least two living tissue sheets, wherein the living tissue sheets are placed in edge contact for a period of time sufficient for causing assembly of the living tissue sheets into a single continuous tissue construct.

[0008] The living tissue sheets used in the preparation of the single continuous tissue construct can be composed of homologous or heterologous types of cells.

[0009] The cell population may comprise at least one type of cells.

[0010] The living tissue sheet comprises at least one cell layer.

[0011] The living tissues can be separated by a separator. The separator can be impermeable or allows selective passage of components contained in a culture medium.

[0012] The contact of cell populations of step b) may be caused by removal of a separator between the at least two living tissue sheets, or by placing the living tissue sheets in contact.

[0013] Another object of the present invention is to provide for producing a tubular tissue construct comprising rolling the continuous tissue construct as described above.

[0014] In accordance with the present invention there is provided a tubular tissue construct that is a blood vessel.

[0015] Another object of the present invention is to provide a method to rapidly produce a living tissue sheet with two or more distinct domains or cell populations, which are perfectly fused or biologically joined together.

[0016] In accordance with the invention, there is also provided a method allowing the creation of leak-proof compartments in a tissue culture flask. Each of these compartments can be separately seeded with different cell types and extracellular matrix, resulting in the creation of a composite sheet of tissue that can be folded, stacked, cut, or rolled in order to create the desired tridimensional tissue constructs. A silicone or rubber separator device that is placed in the culture dish before the seeding of the cells can be used to achieve the separation between the different cell populations. After the cells have attached to their respective part of the dish surface (1 to 24 hours, depending on the cell type), the separator is removed and the cell populations are allowed to grow and close the gap between them. The composite cell sheet that is formed by this method is completely devoid of any suturing artifacts and its different parts are perfectly fused together. The shape of the separator can be designed to allow a particular motif of cell layers to be produced for further assembly in a tridimensional construct. Many separators may be used to generate multiple cell domains on the sheet.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1 illustrates the schematic representation of the method of preparing tissue constructs according to one embodiment of the present invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0018] In many cases, tissue constructs may require that different sheets made of different cell types be assembled together without any suturing technique. This can be achieved by co-culturing the sheets side by side, allowing the cells to reorganize a matrix between them and to make them fused together. Prior to the seeding of the cells, a separator that will create a temporary physical barrier between the flask compartments is placed in the flask.

[0019] The separator can be used with any flask suitable for cell culture. Its design can be made to fit any size and any shape.

[0020] This process can be extended to create more than two domains in a sheet by using many separators. The separators can be of different shape and size and can also be shaped to generate a desired pattern.

[0021] Another embodiment of the present invention is that the continuous tissue construct can be prepared by placing in edge contact at least two cell populations or tissue domains following separated culture of each population or domain.

[0022] Because the first cell culture chamber is separated from the second cell culture chamber, may be by an impermeable or a filtration membrane, a transfer of the cell products from the first cell culture chamber may be avoided or possible without fear of contaminating one or the other cell cultures. The transfer of cell products from one cell culture chamber to the second cell culture chamber can occur continuously or temporarily. The invented culture tissue constructs permits simultaneous culturing of the two cell cultures side by side, for example, in a common culture medium. As the filtration membrane is

impermeable to cells the contamination of the cell cultures with cells of the other cell culture is excluded. After a certain period of time, the separator is removed or put in contact to allow edge fusion of cell populations or living tissue sheets formed by each cell population to give a continuous tissue construct. A matrix can be synthesized at the line of contact of the tissue sheets before and/or during the fusion of the cell populations. The matrix is generally autoproduced by at least one of the cell populations, and can be composed of different collagen types, fibronectin, or of any other compound produced by the cells that will facilitate the tissue sheets fusion.

[0023] The steps of forming cellular populations of the tissue construct may be conducted in the presence of growth factors effective to promote proliferation of the cultured cells employed to repopulate the matrix. For example, but without limitation, when fibroblast cells are employed, a growth factor for use herein may be fibroblast growth factor (FGF), most preferably basic fibroblast growth factor. Epithelial growth factors or other cell proliferation and differentiation factors can be used in the preparation of a continuous tissue construct of the present invention.

[0024] The fibroblast growth factors (heparin-binding) are a family of mitogens active on mesenchymal cells. FGFs are not detected free in conditioned medium, instead the FGFs are found in the extracellular matrix in association with heparin sulfate, localizing in the fibronectin-heparin layer prebound to the transplant tissue matrix. The glycosaminoglycans stabilize FGF activity and are required for FGF binding to cell surface receptors where they stimulate autocrine/paracrine growth.

[0025] One embodiment of the invention uses autologous cells in the process described herein. A tissue sample is taken from the recipient prior to transplant or implant surgery. The cells are taken and cultured in accordance with the methods described herein, to produce continuous tissue construct containing fibroblasts or other cells which are then used to synthesize the allogeneic or xonogeneic tissue construc, in accordance with this process.

[0026] The cell source can be selected to match the tissue to be transplanted. For example, if a blood vessel is to be transplanted, cells can be

taken from a recipient's healthy blood vessel and used as the source of cells for tissue construct preparation. In this fashion, the healthy tissue construct can be very closely matched to the recipient's diseased tissue.

[0027] This aspect is important when the transplant recipient is highly allergic, or if the tissue is highly immunogenic, such as endothelial cells with respect to transplantable blood vessels.

[0028] Among cell types that can be considered, but without limitation, to perform the invention, muscle cells, fibroblasts, and mesenchymal cells can be used. Preferably, muscle cells are smooth muscle cells. Precursor cells can also be used.

[0029] Alternatively, allogeneic cell lines which are not likely to cause an unacceptable immune response upon implant may be used to prepare the tissue construct. Cells with no more than a weak or tolerable allergic response may be used to repopulate the tissue construct to provide a substantially non-immunogenic new or replacement tissue. These cells may be naturally weakly immunogenic or be designed by virtue of recombinant cell technology to be weakly immunogenic.

[0030] In one embodiment of the present invention, the continuous tissue construct can be prepared by placing in culture at least two living tissues or cohesive cell populations in manner to favor the edge contact and eventually the fusion of the tissue sheets for forming one tissue construct.

[0031] The present invention will be more readily understood by referring to the following examples which are given to illustrate the invention rather than to limit its scope.

EXAMPLE I

Apparatus and method for preparing continuous tissue construct

[0032] Fig. 1 depicts the structure of a typical separator. A rigid rod, that can be made of wood, polymer, metal or biological material may be concealed in an elastomer matrix that is shaped in a way that allows the gap between the cells

to be sufficiently small to be covered by the proliferating cells, preferably a blade-like structure with a sharp edge. The assembly has to be made of materials that can be sterilized. At both ends of the rod, a small piece of elastomer functions as a compression fitting that allows the separator to fit tightly in place between the flask walls. When the separator is in place, the edge is in contact with the plastic surface of the culture flask effectively separating the surface in two leak proof compartments. The different cell lines are then seeded in each compartment, and allowed to attach to the surface of the flask before the separator is removed. More than two separators can be used simultaneously to obtain more than two different compartments. This attachment can take 1 to 24 hours, depending on the cell line used (4 hours works well with fibroblasts). The advantage of using a sharp edge on the separator is that only a small gap is created in the cell distribution. As soon as the separator is removed, the growing cells quickly fill this space, resulting in a continuous cell distribution. The sheet of extracellular matrix secreted by these cells will be continuous, allowing the assembly of both cell types into a continuous sheet in one step.

[0033] The present invention was applied on the assembly of a tissue-engineered blood vessel. Natural blood vessel walls are formed of two structurally and functionally distinct layers: the inner contractile media, composed of smooth muscle cells (SMC), and the mechanically strong adventice, composed of fibroblasts. In order to reconstruct this structure, a sheet of SMCs can be rolled over a solid mandrel to form the contractile media layer and a fibroblast sheet is rolled over the first sheet to produce the adventitia layer.

[0034] With the proposed invention, the two cell sheets are made as one long continuous sheet composed of two parts: a SMC domain and a fibroblast domain. The size and length of the sheet can be adjusted by selecting appropriate culture flasks. This longer sheet is rolled in the same manner as the two shorter ones, producing in a single step a complete vessel wall with a dual layer design that mimics the arrangement of the natural vascular wall. The following cell culture methods were used in order to obtain cell sheets: Typically,

a removable separator was placed in a 500 cm^2 culture dish in order to produce two distinct compartments. Viable sub-cultured smooth muscle cells and fibroblasts (passages 3-7) were then separately seeded on each side of the separated culture dish with a final seeding density of 10^4 cells/cm^2. Cells were fed with 80 ml of culture medium (3:1 mixture of Dulbecco's Modification of Eagle's Medium and Ham's F12 Modified Medium, 10% Fetal Clone II from Hyclone, 100 U/ml of penicillin G and 25 ug/ml of gentamicin). The culture medium was changed every two days. A freshly prepared solution of ascorbic acid was added every day to the culture medium at a final concentration of 100 ug/ml. Cells were kept in a humidified atmosphere (92% air and 8% CO_2). Under these conditions the cells will adhere to the culture surface and proliferate until the entire culture surface is covered with cells. The separator has to be in place during the cell adhesion phase and can be removed during the cell proliferation phase or during the sheet formation phase. If the culture conditions are maintained, the cells will grow as a multilayer of cells and endogenous fibrous material and the sheet formed will eventually detach itself as a whole from the substratum. To roll the living sheet, one edge of the sheet is placed between a tubular support and a thread. The thread squeezes the sheet and holds it in place while the sheet is rolled around the tubular support and finally the sheet is re-secured with a thread to prevent unrolling. Thereafter, the tubular living tissue can be cultured for several weeks, with ascorbic acid, to allow further maturation of the tissue.

[0035] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for assembling living tissue sheets for forming a continuous tissue construct comprising the step of:

a) providing at least two cell populations capable of forming at least two separated living tissue sheets, said cell populations being partially or totally confluent; and

b) causing edge contact between said cell populations of step a) for a period of time sufficient for assembling said living tissue sheets into a single continuous tissue construct.

2. The method of claim 1, wherein said cell populations of step a) are composed of homologous or heterologous types of cells.

3. The method of claim 2, wherein said types of cells are mammalian cells.

4. The method of claim 2, wherein said types of cells are selected from the group consisting of mesenchymal cells, muscle cells, or fibroblasts.

5. The method of claim 4, wherein said muscle cells are smooth muscle cells.

6. The method of claim 1, wherein each cell population comprises at least one type of cells.

7. The method of claim 1, wherein said living tissue sheet comprises at least one cell layer.

8. The method of claim 1, wherein said living tissues are separated by a separator.

9. The method of claim 8, wherein said separator is impermeable or allows selective passage of components contained in a culture medium.

10. The method of claim 1, wherein said contact of step b) is caused by removal of a separator between the at least two living tissue sheets, or by placing said living tissue sheets in contact.

11. A method for producing a tubular tissue construct comprising rolling the continuous tissue construct of claim 1.

12. The method of claim 11, wherein said tubular tissue construct is a blood vessel.

13. A single continuous tissue construct composed of at least two living tissue sheets, wherein said living tissue sheets are placed in edge contact for a period of time sufficient for causing assembly of said living tissue sheets into a single continuous tissue construct.

14. The single continuous tissue construct of claim 13, wherein said living tissue sheets are composed of homologous or heterologous types of cells.

15. The single continuous tissue construct of claim 14, wherein said types of cells are mammalian cells.

16. The single continuous tissue construct of claim 14, wherein said types of cells are selected from the group consisting of mesenchymal cells, muscle cells, or fibroblasts.

17. The single continuous tissue construct of claim 16, wherein said muscle cells are smooth muscle cells.

18. The single continuous tissue construct of claim 13, wherein each living tissue sheet comprises at least one type of cells.

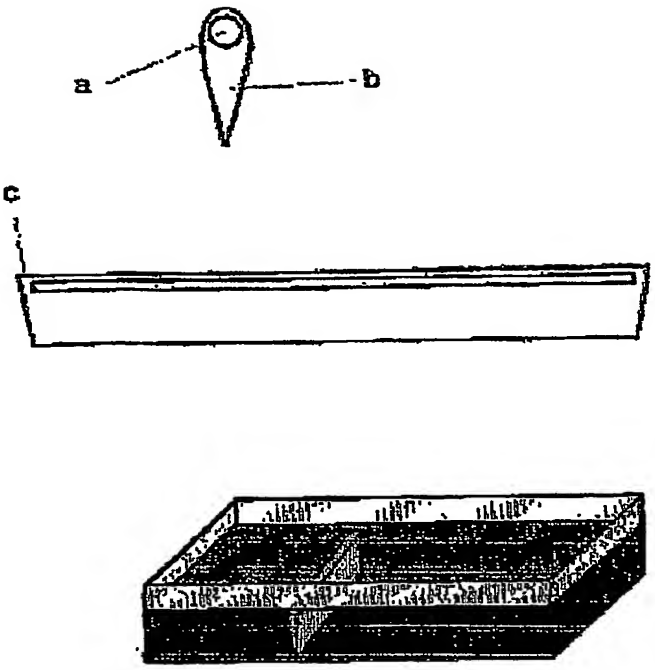
19. The single continuous tissue construct of claim 13, wherein said living tissue sheet comprises at least one cell layer.

20. The single continuous tissue construct of claim 13, wherein said living tissues are placed in edge contact after removal of a separator.

21. The single continuous tissue construct of claim 20, wherein said separator is impermeable or allows selective passage of components contained in a culture medium.

ABSTRACT OF THE DISCLOSURE

The present invetion relates to a method of assembling a single living tissue sheet formed of different cell populations. According to this invention at least two cell populations composing at least two living tissue sheets can be assembled into a single continuous tissue construct. The cells of this construct can also be autologous or heterlogous depending on the needs and the type of implant to be transferred in a recipient patient.



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